EXHIBIT 8

1: Protein Expr Purif. 2005 Jul 19; [Epub ahead of print]

Baculovirus expression, purification, and characterization of human protein phosphatase 2A catalytic subunits alpha and beta.

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Protein phosphatase 2A (PP2A) contains a 36-kDa catalytic subunit (PP2Ac), a 65-kDa structural subunit (PR65/A), and a regulatory B subunit. The core enzyme consists of the structural and catalytic subunits. The catalytic subunit exists as two closely related isoforms, alpha and beta. Several natural toxins, including okadaic acid (OA) and microcystins, specifically inhibit PP2A. To obtain biologically active recombinant PP2A and to compare the properties of the PP2A catalytic subunit alpha and beta isoforms, we expressed human PP2Acalpha and cbeta in High Five insect cells. The recombinant PP2Acalpha and cbeta possess similar phosphatase activities using p-NPP and phosphopeptide as substrates and are strongly inhibited by OA and microcystin-LR to similar degrees. In addition, PP2Acalpha or cbeta was co-expressed with PR65/A and co-purified as a core dimer, PP2A(D) (Aalpha/calpha and Aalpha/cbeta) with PR65alpha/Aalpha. The recombinant PP2A(D) bound to the B subunit in vitro. These results show that the recombinant PP2Acalpha and cbeta are identical in their ability to associate with the A and B subunits, in their phosphatase activities, and in carboxyl-methylation. Furthermore, our results show that High Five insect cells can produce biologically active recombinant PP2A, which should be a valuable tool for detecting natural toxins and investigating the mechanism of PP2A catalysis and other protein interactions.

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